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Time is of essence; rapid identification of veterinary pathogens using MALDI TOF

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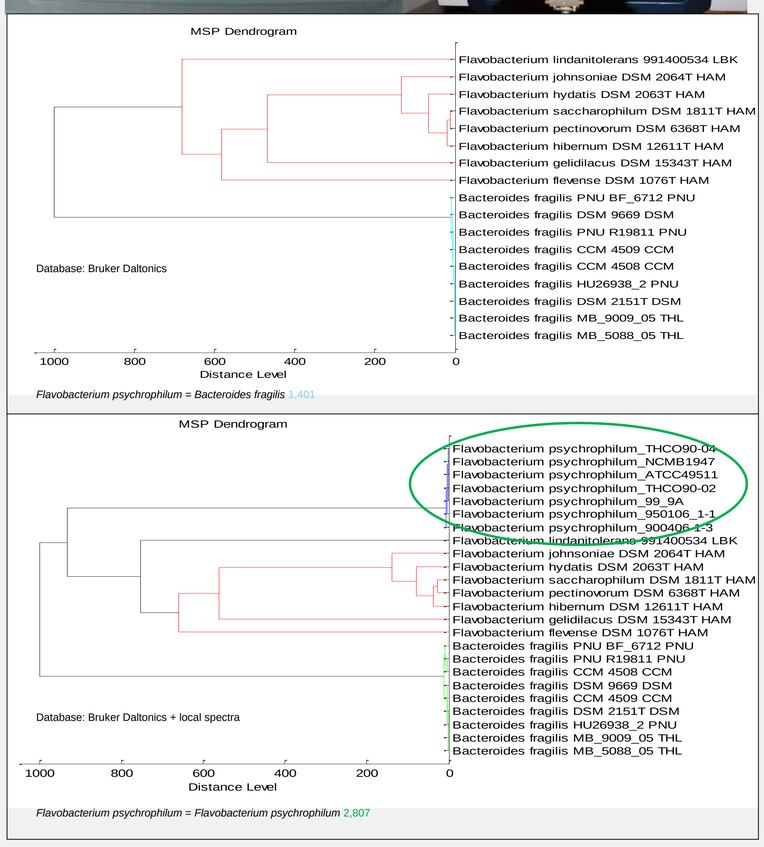
Rapid and accurate identification of microbial pathogens is a cornerstone for timely and correct treatment of diseases of livestock and fish. The utility of the MALDI-TOF technique in the diagnostic laboratory is directly related to the quality of mass spectra and quantity of different microbial species in the database. Since commercial MALDI-TOF spectral database providers mainly focus on human pathogens there is a need for improving the datasets in order to extend the applicability of the technique to the veterinary field. Here we report upgrading of a commercial MALDI-TOF database with the mass spectra of fish and mastitis pathogens as well as pathogens relevant for surveillance of diseases in farm animals and wildlife.

Aim

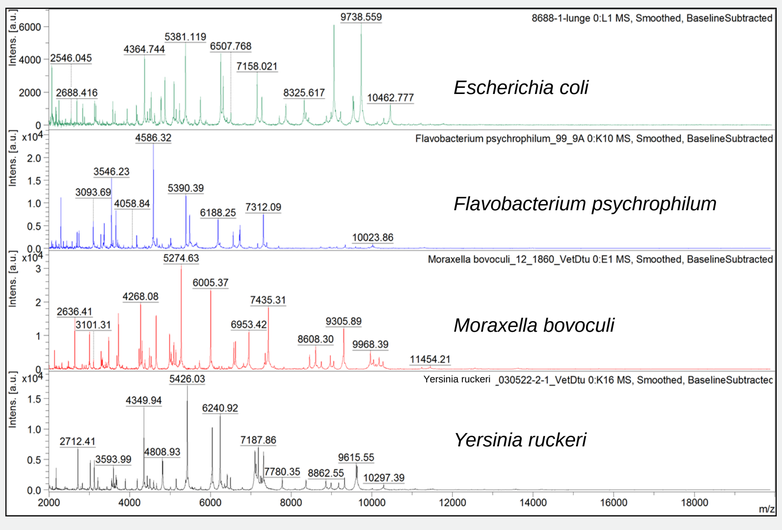
To obtain spectral coverage of a given species, preferably, with af minimum of 5 spectra for each species.

Method

All field isolates used as references in the local database were identified by conventional diagnostics and biochemical test. (PCR or sequencing). Isolates were subjected to the Bruker formic acid/ acetonitrile extraction procedure with minor alterations.Spectra were obtained using Flexcontrol version 3.4 at an Autoflex Speed, (Bruker Daltonics, Germany). Analysis and establishment of new local reference spectra were achieved with Flexanalysis 3.4 and Biotyper 3.1 software.



Before and after database supplement: an isolate identified as *Flavobacterium psychrophilum* was subjected to MALDI-TOF identification. Log score values above 2.0 indicate identification at species level. Submission only to the database by Bruker Daltonics provided a low score below the species identification (1.6) suggesting *Bacteroides fragilis.* After local reference spectra were achieved, submission of the same isolate to the database extended with local spectra, a species identification of 2.8 was obtained for *F. psychrophilum*.



The upgraded spectral database has been extensively evaluated for identification of fish pathogens (*Aeromonas*, *Vibrio*, *Yersinia* and *Flavobacterium*) and to less extent for identification of mastitis bacteria and pathogens of wildlife.

Perspectives

Further work is underway to improve quality of the database and to extend the applicability of the technique to identification at the sub-species level (microbial typing).

Results and Conclusion

Results = Day one of pure culture, cost ≈ 1 €, time 15 minutes

All of the obtained mass spectra were of sufficient quality to allow unambiguous differentiation of the tested bacteria so the local database was upgraded with the following species: *Aeromonas caviae* (n=1), *Aeromonas salmonicida* (n=3), *Vibrio anguillarum* (n=16), *Vibrio ordalii* (n=1), *Yersinia ruckeri* (n=3), *Flavobacterium psychrophilum* (n=7), *Streptococcus canis* (n=4), *Streptococcus bovis* (n=1), *Micrococcus luteus* (n=1), *Moraxella bovis* (n=1*), Moraxella bovoculi* (n=2), *Pasteurella aerogenes* (n=2), *Pasteurella canis* (n=2), *Pasteurella dagmatis (n=1) Pasteurella langaa (n=1), Pasteurella mairii (n=1),Staphylococcus chromogenes* (n=5), *Streptococcus agalactiae* (n=5) and *Taylorella equigenitalis* (n=3).

In all cases there was an apparent improvement of Biotyper scores for identification at the species level and a significant reduction of time and cost from pure culture to diagnostic result at species level.

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